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Bridget K. Carr

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Congenital Limb Reduction Defects in Infants:

A Look at Possible Associations With Maternal Smoking and Hypertension

by

Bridget K. Carr

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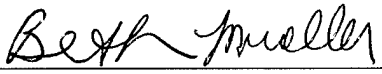
Approved by 

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Abstract

Congenital Limb Reduction Defects in Infants:
A Look at Possible Associations With Maternal Smoking and Hypertension

by Bridget K. Carr

Chairperson of the Supervisory Committee:

Associate Professor Beth Mueller

Department of Epidemiology

Case control studies from Finland, Sweden, and Hungary suggest an association between prenatal smoking and infant limb defects possibly due to vascular disruption in the embryo. It has also been suggested that maternal hypertension may be associated with the occurrence of congenital limb defects, either due to the effects of the disease or possibly medications such as the calcium channel blockers used to treat this condition. This project examined these associations using the Birth Events Records Database (BERD) which links Washington state birth certificate data with birth hospitalization records. Between 1987 and 1995, 1544 singleton infants with limb defects [syndactyly (N=489), polydactyly (N=866), and reduction defects (N=189)] were identified by ICD-9 codes contained in the BERD file. These cases were frequency-matched by year of birth to 6176 children without congenital limb defects. Prenatal smoking was found not to be associated with an increased risk of limb defects in general (odds ratio [OR]=1.06, 95% confidence interval [CI] 0.91, 1.25), nor were the presence of either gestational hypertension (OR=1.12, CI = 0.85, 1.48) or chronic hypertension (OR=1.02, CI=0.57, 1.84), as indicated on the birth certificate, associated with increased risk of giving birth to

children with limb defects. However, examination of specific types of malformation revealed that maternal smoking was associated with a greater than two-fold increased risk of transverse reduction limb defects (OR=2.18, CI=1.13, 4.20). Chronic or gestational hypertension were not associated with increased risks of any specific defect. This finding related to smoking is consistent with previous studies, and emphasizes the continued need for public health interventions designed to reduce prenatal smoking. Studies including a larger number of hypertensive women, preferably including data on the use of specific anti-hypertensive medications prenatally, are needed before any conclusions can be drawn between possible associations of maternal hypertension (or its treatment) and the occurrence of congenital limb defects.

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INTRODUCTION

The desire to have healthy children is one of the most treasured aspects of life. For those seeking parenthood, having a "perfectly formed" child is virtually a universal wish. Congenital limb reduction defects have a birth prevalence of 5-10 per 10,000(3) and are often accompanied with other anomalies. Infants with limb reduction defects have an 18% increased risk of early death and often require a median of 5 hospitalizations, costing $22,000 per child-year for the first 2 years of life(14,30). Most congenital birth defects have unknown causes. Genetic and environmental factors have been associated with the occurrence of many congenital anomalies. Ingestion of tobacco has caused congenital limb deformities in swine(22) and cattle(18), and maternal smoking has previously been associated with limb deformities in human infants(2,5,17). Vascular disruption during embryogenesis has been hypothesized as the mechanism leading to some limb defects(5,6,11,15).

Prevalence of maternal smoking decreases during pregnancy, compared to pre-pregnancy(12,13,26). Smoking during pregnancy has been identified as a potential risk factor for congenital limb reduction defects (CLRD; absence or shortening of digits or limbs). To date, no population based studies of the risk of CLRD among infants born to women who smoked during pregnancy have been reported from United States data, although previous studies demonstrating this risk have been conducted in Hungary(5) and Sweden(17). The former concluded that maternal smoking during pregnancy raises the relative odds for terminal transverse limb deficiencies, (odds ratio [OR]=1.48; 95% confidence interval [CI]=0.98, 2.23) and raises the relative odds for amniogenic limb deficiencies (OR=1.62; CI=1.05, 2.52).

Chronic hypertension and/or anti-hypertension medications have been suggested as potential risk factors for CLRD. In theory, hypoperfusion of the fetus could result from uncontrolled maternal hypertension, or anti-hypertension medication could interfere with apoptosis (programmed cell death) in the fetal limb, either of which could lead to abnormal development of fetal extremities.

The purpose of this project was to explore the risk of giving birth to infants with congenital limb defects (particularly reduction types) associated with prenatal smoking, and chronic or gestational hypertension. Identifying an association between limb reduction malformations and these relatively common exposures among pregnant women is of great public interest. Knowledge of these risks would be useful for pre-conception counseling and prenatal care for women who are hypertensive or who smoke during pregnancy.

METHODS

A population based case control study was conducted using data from the Washington State Birth Events Records Data Base (BERD) for the years 1987-1995. This file, created by the Washington State Department of Health, contains hospital discharge data for the mother and child for the birth hospitalization linked to data from the birth certificate. Cases were all singleton live born infants with congenital limb deformities receiving an ICD-9 code of 755.0-755.40 in the BERD (N = 1544). For comparison, four infants per case without these deformities were randomly chosen from among the same year of birth (N = 6176). Maternal age, paternal age, maternal prenatal smoking history, maternal hypertension, and other infant and maternal characteristics were obtained from the birth certificate. Mothers were classified as smokers if their records indicate having smoked at any time during pregnancy. Hypertension status (both gestational and chronic) was indicated on the birth certificate.

The classification of limb defects follows the International Classification of Disease 9th revision(16). These are broadly classified into polydactyly (755.0), syndactyly (755.1) and reduction deformities (755.2-755.4). Polydactyly is the presence of more than five digits on either the hand or foot. Synonyms include hyperdactylia, hyperdactylism, hyperdactyly, polydactylia, polydactylism, and supenumerary digits. Syndactyly is webbing or fusion of the toes or fingers, and can occur with or without bone involvement. Synonyms include aschistodactyly, aschistodactylia, dactylia, dactylium, syndactylia, syndactylism, symphalangism and symphalangy. Reduction deformities or congenital shortening can also occur in either the upper (755.2) or lower (755.3) limb (unspecified limb code is 755.4). This deformity may be of an unspecificed type (755.20, 755.30), including ectromelia, or gross hypoplasia, or aplasia of

one or more of the long bones. This would include amelia (a congenital absence of an entire limb), hemimelia (the absence of all or part of the distal half of the limb), meromelia (absence of any part of a limb), or phocomelia (absence of the proximal portion of a limb) with the hand or foot attached by a small bone. Transverse deficiencies (755.21, 755.31) include absence of the entire limb. For example in the upper limb, it would include absence of all fingers (complete or partial), absence of the forearm (including hand and fingers), congenital amputation of upper limb, and transverse hemimelia (absence of all or part of the distal half of the limb). The remainder of the reduction defects are classified as longitudinal deficiencies (755.22-755.29, 755.32-755.39). These conditions include phocomelia, and absence of the humerus, radius, ulna, femur, tibia, fibula, metacarpals or metatarsals with some distal elements remaining, as well as complete or partial absence of toes or fingers (excluding terminal deficiency) of all five digits, and transverse deficiency of phalanges.

Infants were classified by the first limb defect indicated in a total of several fields possible (5 fields possible for 1987-1991; 9 fields possible for 1992-1995). Thus, an infant may have had more than one defect. In general, completeness of exposure data on birth certificates improved after 1991. Overall, smoking status was unknown for 10.7% of cases and 6.1% of controls; hypertension status was unknown for 9.4% of cases, and 7.3% of controls. All subjects with missing or unknown data for smoking or hypertension were excluded from the respective analysis of each exposure. Stratified analysis was conducted and Mantel-Haenszel odds ratio estimates are presented where appropriate. Variables considered as potential confounders and/or effect modifiers included: prenatal alcohol consumption (ever, never), maternal and paternal age (<20, 20-29, 30+ years), parity (0, 1, 2+ prior births), gravidity (0, 1, 2, 3+ prior pregnancies),

diabetes (yes [established or gestational], no), marital status (married, unmarried) and race (White, Black, Other).

RESULTS

Of the 1544 infants with limb deformities, 866 had ICD-9 codes for polydactyly, 489 for syndactyly, and 189 with limb reduction-type defect codes. Of the latter, 46 were coded as a transverse reduction and 121 as a longitudinal reduction. The remaining 22 (of 189) were otherwise unspecified reduction defects. 1317 (85.3%) of the case infants were without ICD-9 codes for other malformation, thus considered to have isolated defects. Of the limb reduction defects, 128 of 189 (67.7%) were isolated; of the transverse type of reduction defect, 37 of 46 (80.4%) were isolated.

Case and control parents did not differ significantly in age, although case mothers and fathers were slightly younger than controls (Table 1). Gravidity and parity were generally similar for case and control mothers, however 17.5% of case mothers were Black, compared to 3.8 % of controls. This discrepancy was largely due to a disproportionately greater number of black infants with polydactyly. Hypertension, diabetes, and smoking histories were similar in case and control mothers. A larger proportion of case mothers (32.5%) than control mothers (24.5%) were unmarried. Case infants were more likely to be male (62.4%) than female, while gender of control infants was more evenly distributed (50.8% male).

Prenatal smoking was not found to be associated with an increased risk of limb defects in general (mother's race, marital status and gravidity adjusted OR=1.06, CI=0.91, 1.25; Table 2), nor were the presence of either gestational hypertension (smoking adjusted OR=1.08, CI=0.81, 1.44) or chronic hypertension (smoking adjusted OR=1.03, CI=0.57, 1.84), as indicated on the birth certificate, associated with increased risks. However, when specific limb reduction malformation

groups were evaluated, maternal smoking was associated with a greater than two-fold increased risk of transverse reduction limb defects (mother's race, marital status and gravidity adjusted OR=2.17, CI=1.13, 4.20; Table 3). This association was not affected by adjustment for paternal age, number of prior births, presence of maternal diabetes or hypertension, prenatal alcohol use or prenatal drug use. A sub-analysis of the relationship between maternal smoking and isolated limb reduction defects (N=35) found a similar estimate (mother's race, marital status and gravidity adjusted OR=2.19, CI=1.07, 4.48). Mothers who had chronic hypertension indicated on their birth certificates had a slightly elevated risk of having an infant with polydactyly, however, this could have occurred by chance.

Table 1. Selected maternal, paternal and infant characteristics of births resulting in limb defects for all cases, case subgroups, and comparison births without limb defects (controls).

PROPORTION OF LIMB DEFECT CASES AND CONTROLS AND THEIR CHARACTERISTICS

CHARACTERISTIC	All Cases N = 1544 # (%)	Polydactyly N = 866 # (%)	Syndactyly N = 489 # (%)	Reduction N = 189 # (%)	Control N = 6176 # (%)
Maternal age, yrs					
<20	205 (13.3)	130 (15.1)	48 (9.8)	27 (14.3)	647 (10.5)
20-29	844 (54.7)	469 (54.2)	266 (54.5)	109 (57.7)	3408 (55.2)
30+	494 (32.0)	267 (30.8)	174 (35.7)	53 (28.0)	2119 (34.3)
Paternal age, yrs					
<20	168 (12.7)	119 (16.9)	32 (7.1)	17 (9.9)	512 (9.2)
20-29	571 (43.1)	286 (40.5)	200 (44.6)	85 (49.4)	2357 (42.5)
30+	587 (44.3)	301 (42.6)	216 (48.2)	70 (40.7)	2684 (48.3)
Prior pregnancies					
None	500 (32.4)	270 (31.2)	157 (32.1)	73 (38.6)	1939 (31.4)
One	428 (27.7)	237 (27.4)	148 (30.3)	43 (22.8)	1737 (28.1)
Two	267 (17.3)	147 (17.0)	82 (16.8)	38 (20.1)	1176 (19.0)
Three or more	349 (22.6)	212 (24.5)	102 (20.9)	27 (18.5)	1324 (21.4)
Prior births					
None	671 (44.1)	369 (43.1)	208 (43.3)	94 (50.8)	2531 (41.6)
One	462 (30.4)	263 (30.7)	152 (31.7)	47 (25.4)	1964 (32.3)
Two or more	388 (25.1)	224 (26.2)	120 (25.0)	44 (23.8)	1594 (26.2)
Maternal race					
White	1041 (69.8)	475 (56.9)	413 (87.3)	153 (83.2)	4911 (81.5)
Black	261 (17.5)	238 (28.5)	14 (3.0)	9 (4.9)	226 (3.8)
Other	190 (12.7)	122 (14.6)	46 (9.7)	22 (12.0)	892 (14.8)
Infant sex					
Male	963 (62.4)	522 (60.3)	336 (68.7)	105 (55.6)	3137 (50.8)
Female	581 (37.6)	344 (39.8)	153 (31.3)	84 (44.4)	3039 (49.2)
Maternal diabetes					
Yes	38 (2.7)	21 (2.7)	10 (2.3)	7 (4.0)	132 (2.3)
No	1361 (97.3)	765 (97.3)	427 (97.7)	169 (96.0)	5594 (97.7)
Marital Status					
Married	1040 (67.5)	523 (60.5)	386 (79.3)	131 (69.3)	4653 (75.5)
Unmarried	501 (32.5)	342 (39.5)	101 (20.7)	58 (30.7)	1510 (24.5)

Table 2. Estimated risk of congenital limb defects associated with prenatal smoking, gestational hypertension and chronic hypertension.

	EXPOSURE		
SUBJECTS	**PRENATAL SMOKING**	**GESTATIONAL HYPERTENSION**	**CHRONIC HYPERTENSION**
CONTROLS (N=6176)			
Proportion exposed	19.2%	4.4%	1.1%
ALL DEFECTS (N=1544)			
Proportion exposed	21.4%	4.6%	1.2%
OR[1] (95% CI)	1.06 (0.91, 1.25)	1.08 (0.81, 1.44)	1.03 (0.57, 1.84)
POLYDACTYLY (N=866)			
Proportion exposed	22.5%	5.6%	1.7%
OR[2] (95% CI)	1.05 (0.85, 1.30)	1.3 (0.94, 1.81)	1.54 (0.85, 2.78)
SYNDACTYLY (N=489)			
Proportion exposed	19.2%	3.9%	0.9%
OR[3] (95% CI)	1.14 (0.87, 1.50)	0.78 (0.45, 1.34)	0.84 (0.31, 2.33)
REDUCTION (N=189)			
Proportion exposed	22.2%	3.8%	0.5%
OR[1] (95% CI)	1.10 (0.76, 1.58)	0.98 (0.45, 2.10)	0.57 (0.08, 4.04)

[1]Prenatal smoking OR is adjusted for marital status and gravidity; chronic and gestational hypertension ORs are adjusted for maternal smoking.
[2]Prenatal smoking OR is adjusted for race, gravidity and marital status.
[3]Prenatal smoking OR is adjusted for marital status and paternal age; chronic and gestational hypertension ORs are adjusted for maternal smoking.

Table 3. Estimated risk of transverse and longitudinal type of reduction limb defects associated with prenatal smoking, gestational hypertension, and chronic hypertension.

	EXPOSURE		
SUBJECTS	**PRENATAL SMOKING**	**GESTATIONAL HYPERTENSION**	**CHRONIC HYPERTENSION**
CONTROLS (N=6176)			
Proportion exposed	19.2%	4.4%	1.1%
TRANSVERSE (N=46)			
Proportion exposed	35.7%	4.4%	0%
OR* (95% CI)	2.18 (1.13, 4.20)	1.02 (0.25, 4.28)	1.00 (0.06, 16.35)
LONGITUDINAL (N=121)			
Proportion exposed	20.4%	3.6%	0.9%
OR (95% CI)	1.18 (0.68, 1.71)	0.83 (0.30, 2.27)	1.54 (0.85, 2.78)

*Prenatal smoking OR adjusted for race, gravidity and marital status.

DISCUSSION

Prenatal smoking was not generally associated with an increased risk of congenital limb defects in offspring. When specific types of defects were examined, a greater than two-fold increased risk of transverse reduction defects associated with smoking was observed. This is consistent with prior studies in Hungary(5) and Sweden(17). The study in Hungary concluded maternal smoking during pregnancy raises the odds for terminal transverse limb deficiencies (OR=1.48; CI=0.98, 2.23) and raises the odds for amniogenic limb deficiencies (OR=1.62; CI=1.05, 2.52). The exposure assessment in this study was by in person survey conducted a median of 11 months post-partum, and is subject to maternal memory bias. The Swedish study, which was population-based, identified a moderate risk increase for limb reduction defects among infants born to smoking women (OR=1.26; CI=1.06, 1.5). Other studies suggest similar but non-significant risk esti-mates(1,2,7,25) . Other factors associated with increased risk of congenital limb reduction defects in earlier studies include high birth order, diabetes, high maternal age, previous malformations in the family, and possibly alcohol consumption.

The role of maternal smoking during pregnancy in the origin of terminal transverse defects is theoretically plausible as maternal smoking may cause vasospasm, subsequent ischemia, necrosis, and ultimate resorption of structures that are distal to the vascular occlusion in the embryo(5,11,15). An interaction between the apical epidermal ridge and the underlying mesoderm is responsible for normal limb development in the embryo. A direct relationship between the development of this ridge and the vessels of the limb bud has been found. Damage to one may lead to damage of the other(29).

In general, males are more commonly affected with limb defects, particularly those defects with familial association(3,6,29). Among the etiological explanations of transverse defects (with characteristic sporadic or non-genetic and monomelic involvement), the vascular disruption hypothesis described by Czeizel and colleagues(5,6) seems most probable. They hypothesized that transverse defects are usually secondary to an intrauterine vascular accident leading to interruption of blood supply to the developing structure, thus a lack of familial clustering, and sex predilection are expected(3,6). In this study, male sex was associated with case status for all limb defects. This was an expected finding, as 1355 (88%) of the cases were polydactyly or syndactly, both of which have a significant familial association. Though males with transverse defects outnumbered females (28:18), the difference is one not unexpected by chance. Further, transverse types of congenital limb deficiency are not usually associated with non-limb defects, and typically only one limb is affected. In this study, cases were identified as having isolated defects if no other malformations were listed in the ICD-9 codes contained in the BERD file. By this method, 1317 of 1544 (85.3% of total cases) were classified as having isolated defects, and 37 of 46 (80%) of the subgroup of transverse reduction defects were classified as isolated.

This study also examined the association between maternal gestational or chronic hypertension and the risk of delivering an infant with a congenital limb defect. From 1987 to 1995, only 80 women with chronic hypertension and 317 women with gestational hypertension were identified from birth certificate data. No association was found between gestational hypertension and all limb defects or between gestational hypertension and specific subcategories of limb defects. Neither was an association found between chronic hypertension and all limb defects or any of the specific subcategories of limb defects.

Arterial hypertension is a condition of sustained elevated systemic arterial blood pressure usually involving elevations in mean and pulse pressures. The minimum level of systemic arterial pressure considered to be hypertensive has been arbitrarily set at 140/90 mm Hg(24). Pregnancy can not only induce hypertension, it can intensify already existing hypertension.

Placental blood flow is dependent on maternal blood pressure rather than the activity of the embryonic heart(29). Therefore, hypertension could result in congenital limb defects through vasocostriction, increased peripheral resistance, and reduced blood flow to the uterus. Several animal models indicate that vasoconstriction can result in limb defects. Impairment of the uterine blood flow by temporary clamping of the uterine blood vessels for 30-90 minutes in gravid rats induced a high incidence of defects in the distal parts of the limb of offspring(19) In addition, Ugen and Scott(27) demonstrated that both clamping of the uterine vessels in gravid rats, and vasoconstrictive substances (serotonin, ergotamine and nicotine) all potentiate digital defects induced by acetazolamide, a carbonic anhydrase inhibitor. They postulated that these effects were the results of uterine vasoconstriction.

Alternatively, anti-hypertensive drugs could affect fetal circulation indirectly by changes in maternal circulation (hypotension) or directly through placental transfer of the drug. Disruption of utero-placental blood flow by anti-hypertension drugs seems possible but unlikely. The vasodilating drug nicardipine given on day 24-26 of gestation in pregnant rabbits (normal gestation period is 30-35 days) produced a significant increase in maternal cardiac output but a fall in utero-placental perfusion. The authors noted that the question still remained whether the observed decrease in utero-placental perfusion was significant enough to interfere with fetal well being(20). In addition, the change in systolic/diastolic ratio (SD) in 8 primigravid human

patients experiencing acute gestational hypertension before and after intravenous administration of nicardipine (a calcium channel blocking agent) was measured. There was a transient rise in utero-placental SD 30 minutes after administering the drug, but no change in SD was seen in umbilical blood flow to the fetus. This suggests that if the infant and mother are able to respond to circulatory changes induced by therapy, anti-hypertension agents may not result in adverse changes in fetal blood flow(31). However, Danielsson and colleagues(9,10) found a dose related trend in digital defects of the distal phalanx after administration of vasodilating agents to pregnant rabbits. They concluded that the mechanism of action was secondary to pharmacological action and not related to chemical structure of the drugs since similar defects resulted from compounds with completely different structures (i.e., hydralazine versus three different calcium channel blocking agents: nifedipine, nitrendipine, and felodipine). Decreased uterine blood flow was considered the most probable explanation. These investigators further examined the hypothesis that limb defects were due to decreased utero-placental blood flow and not the result of a direct affect on chondrogenesis. They examined the limb plates in fetal rabbits after maternal administration of felodipine and found histopathology similar to the lesion associated with clamping of uterine vessels. The author noted that rabbits had a very high and sustained plasma concentration of felodipine compared with human therapeutic concentrations, and that observed effects of high does of vasodilators do not seem to be related to the clinical use of vasodilators at therapeutic does in humans.

A third possible mechanism for hypertension associated with limb defects is by the anti-hypertension agent directly affecting the developing embryo(9,10). Calcium channel blocking agents are one class of anti-hypertension medications. They inhibit calcium ion entry across cell membranes and reduce intracellular calcium concentrations in cardiac and vascular smooth

muscle cells(28). Apoptosis, or programmed cell death, is defined by condensation and breakdown of cell to cell contact, fragmentation and phagocytosis by neighboring cells. Apoptosis is a normal physiological process to control cell populations during embryogenesis, immune response, and normal tissue homeostasis. Cell death is particularly prominent in limb development; it results in the shapes and contours of the digital palettes(32). Daling(8) states that in "many cell types a sustained rise in cytosolic ionized calcium initiates the apoptotic process. Calcium is also involved in the activation of the endonuclease enzyme, which leads to DNA fragmentation." It is then conceivable that by inhibiting calcium entry into cells, calcium channel blocking agents could disrupt normal apoptosis in the developing limb, resulting in limb defects. The association of any of these mechanisms and CLRD was not detected this study.

One multicenter cohort study has examined the safety of calcium channel blockers in pregnant women21 Cases were 78 women who contacted any of six teratogen information centers about exposure to any calcium channel blocker. 73% of the women were exposed only during the first trimester. The sample size only had the power to detect a 5 fold increase in birth defects above a baseline rate of 2% (alpha=0.05, beta=0.20). No increase in major malformations were detected. Two limb malformations occurred; they were attributed to maternal diabetes and co-ingestion of teratogens. This left the author to doubt the role of calcium channel blockers as a major teratogenic risk. Because of small sample size, further studies were recommended.

This study's use of Birth Certificate data to broadly explore the association between chronic maternal hypertension and the delivery of an infant with a limb defect was quite reasonable, however missing data and misclassification of chronic hypertension may have been reduced by

also using the BERD. The small sample size of women identified with chronic hypertension (80; 18 with limb defects) would only allow identification of a risk of limb defects which was over 3 times greater among women with hypertension relative to normotensive women (alpha=0.05, beta=0.20). The effective sample size could be considerably smaller when considering only those women with uncontrolled hypertension or those on calcium channel blocking agents during the first trimester of pregnancy. Consequently, finding a lack of an association between chronic hypertension and/or anti-hypertension medications during pregnancy and congenital limb defects should not be considered conclusive. Further studies with larger numbers of hypertensive women and more specific information on maternal medication use are needed to continue exploring the potential relationship of maternal hypertension or calcium channel blocking agents and limb defects in infants.

An association between gestational hypertension and congenital limb defects was not expected. This category of hypertension was included as a comparison group. The type and severity of an induced congenital defect will depend on the dose and type of insult, and the developmental phase of the embryo at the time of injury. The digits of the developing fetus are separate and identifiable by the 8th week of gestation in humans. Gestational hypertension usually occurs after the 20th week of pregnancy(4). By this time limb development is well advanced, pregnancy status is usually known, and treatment for gestational hypertension rarely includes calcium channel blockers(4).

This study was population-based, and thus avoided many of the problem of selection bias. It was limited by the use of birth certificate data which only capture live born infants. Fetal losses and elective termination of pregnancy data were not available, resulting in possible bias. The

incidence of congenital limb defects in those infants or fetuses that are not live born is approximately 6 times greater(29). The loss of such cases would make it more difficult to detect an association if one exists. In birth certificate data, one must be concerned with coding errors, the individual clinician's ability to classify pregnancy complications correctly, and the under-reporting of mild cases, including subtle reduction anomalies(2). The effect of such errors is probably small in this study. Both the visibility of limb defects as well as use of the BERD served to improve identification of limb defects. In addition, it is reasonable to assume that if subtle anomalies were missed, they would be missed in all pairs of exposure categories equally (smoking, non-smoking; hypertensive, non-hypertensive), rather than selectively.

Smoking information was missing from 10.7% of the cases, and 6.1% of the controls. If these cases and controls with missing data differed with respect to smoking and limb defect status of infant, the risks could be biased. It is unlikely that this amount of missing data would significantly change odds ratios for transverse reduction type defects, however underestimation of the risk is more likely than overestimation.

A sub-analysis of isolated limb defects from limb defects occurring with other congenital malformations demonstrated the same risk estimate associated with prenatal smoking. Genetic disorders account for roughly 17% of limb defects(3,29) and many of the remainder show recognized associations. Malformations in other organ systems have been identified in 32% of cases(29), in contrast to 15-20% of reduction defects in this study. It is reasonable to assume that a number of limb defects occurring concurrently with defects in other organ systems are attributable to causes other than smoking or hypertension. The comparison study of isolated limb reduction defects resulting in the same risk estimate associated with prenatal smoking

supports that a genetic association is unlikely the cause of the identified defect risk in this study, and further supports the vascular disruption theory described by Cziezel(5,6).

To date, no population based evaluations of the risk of smoking and congenital limb reduction defects have been reported from United States data. This study identified a greater than two-fold increase risk of transverse type limb reduction defects in infants born to women who smoked during pregnancy as compared to women who did not smoke during pregnancy. This strongly suspected causal effect of maternal smoking is a further indication for public health interventions aimed at preventing smoking during pregnancy.

REFERENCES

1. Aro T, Haapakoski J, Heinonen, OP. A Multivariate Analysis of the Risk Indicators of Reduction Limb Defects. International Journal of Epidemiology, 1984; 13:459-464.
2. Aro T. Maternal diseases, alcohol consumption and smoking during pregnancy associated with reduction limb defects. Early Human Development, 1983; 9:49-57.
3. Cobben JM, Hiemstra S, Robinson PH. Genetic counseling in limb reduction defects. Genetic Counseling, 1994; 5:243-248.
4. Cunningham, MacDonald, Gant, Leveno, Gilstrap, Hankins, and Clark (Editors). Williams Obstetrics. Stanford, Appleton and Lange, 1997.
5. Czeizel AE, Kodaj I, Lenz W. Smoking during pregnancy and congenital limb deficiency. BMJ, 1994; 308:1473-6.
6. Czeizel AE, Kodaj MV, Lenz W. A Morphological and Family Study on Isolated Terminal Transverse Type of Congenital Limb Deficiency in Hungary, 1975-1984. Teratology, 1993; 48:323-327.
7. Czeizel AE, Kodaj MV, Lenz W. An epidemiological study of isolated split hand/foot in Hungary, 1975-1984. Medical Genetics, 1993; 30:593-596.
8. Daling JR. Calcium channel blockers and cancer: Is an association biologically plausible? AJH 1996; 9:713-14.
9. Danielsson BRG, , Reiland S, Rundqvist E, Danielsson M. Digital defects induced by vasodilating agents: relationship to reduction in uteroplacental blood flow. Teratology, 1989; 40:351-358.
10. Danielsson BRG, Danielsson M, Reiland S, Rundqvist E, Denker L, Regard CG. Histological and in vitro studies supporting decreased uteroplacental blood flow as explanation for digital defects after administration of vasodilators. Teratology, 1990; 41:185-193.
11. Froster UG, Baird PA. Amniotic band sequence and limb defects. Data from a population based study. American Journal of Medical Genetics, 1993; 46:497-500.

12. Haug K, Aaro LE, Fuegelli P. Smoking habits in early pregnancy related to age of smoking debut. Family Practice, 1993; 10;66-9.

13. Haug K, Aaro LE, Fuegelli P. Smoking habits in early pregnancy and attitudes towards smoking cessation among pregnant women and their partners. Family Practice, 1992; 9:494-9.

14. Henderson J, Goldacre MF, Fairweather JM, Marcovitch, H. Conditions accounting for substantial time spent in hospital in children aged 1-14 years. Archives of Diseases in Childhood. 1992; 67:83-86.

15. Hoyme EH, Hones KL, Van Allen MI, Saunder BS, Benirschke K. The vascular pathogenesis of transverse limb reductions defects. Journal of Pediatrics, 1982;101:839-843.

16. International Classification of Diseases 9th Revision clinical Modification. Geneva: World Health Organization, 1980.

17. Kallen K. Maternal Smoking during Pregnancy and Limb Reduction Malformations in Sweden. American Journal of Public Health. 1997; 87:29-32.

18. Keeler RF. Congenital defects in calves from maternal ingestion of *Nicotiana glauca* of high anabasine content. Clinical Toxicology, 1980; 15:417-426.

19. Leist KH, Grauwiler J. Fetal Pathology in rats following uterine vessel clamping on day 14 of gestation. Teratoloty, 1974; 10:55-66.

20. Lirette M, Holbrook HR, and Katz M. Cardiovascular and uterine blood flow changes during nicardipine HCl tocolysis in the rabbit. Obstetrics and Gynecology, 1987; 69:79-82.

21. Maagee LA, Schick B, Donnenfeld AE, Sage SR, Conover B, Cook L, McElhatton PR, Schmidt MA, and Koren G. The safety of calcium channel blockers in human pregnancy: A prospective, multicenter cohort study. American Journal of Obstetrics and Gynecology, 1996; 174:823-8.

22. Menges RW, Selby LA, Marienfeld CF, Aue WA, Greer DL. A tobacco Related Epidemic of Congenital Limb Deformities in Swine. Environmental Research, 1970; 3:285-302.

23. Polednak AP, Janerich DT. Maternal factors in congenital Limb-Reduction Defects. Teratology, 1985; 32:41-50.

24. Selkurt EE. Physiology. Boston, Little, Brown and Company, 1976.

25. Shiono PH, Klebanoff MA, Berendes HW. Congenital Malformations and Maternal Smoking During Pregnancy. Teratology, 1986; 34:65-71.

26. Stewart PJ, Potter J, Dulberg C, Niday P, Nimrod C, Tawagi G. Change in smoking prevalence among pregnant women 1982-93. Canadian Journal of Public Health, 1995; 86:37-41.

27. Ugen KE, Scott WJ. Potentiation of acetazolamide induced ectrodactyly in wistar rats by vasoactive agents and physical clamping of the uterus. Teratology 1986; 31:273-278.

28. USP DI, Drug Information for the Health Care Professional. Taunton, Rand M^{C}Nally, 1997.

29. Van Allen MI. Vascular Disruptions in Gilbert-Barness E (editors): Potter's Pathology of the Fetus and Infant. St. Louis, Mosby-Year Book Inc. 1997.

30. Waitzman NJ, Romano PS, Scheffler RM. Estimates of the economic costs of birth defects. Inquiry, Summer 1994; 33:188-205.

31. Walker JJ, Mathers A, Bjornsson S, Cameron AD, and Fairlie FM. The effect of acute and chronic antihypertensive therapy on maternal and fetopoacental doppler velocimetry. European Journal of Obstetrics and Gynecology and Reproductive Biology, 1992; 43:193-199.

32. Zaker ZF, Ahujua HS. Apoptotic cell death in the limb and its relationship to pattern formation. Biochemistry and Cell Biology, 1994; 72:603-13.